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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MEMANTINE AND DONEPEZIL IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT:

For the reason of evaluating memantine and donepezil in tablet dose form simultaneously, a simple, accurate, and precise technique was established. An Agilent 150mm 4.6mm 5µ filter was used to perform a chromatogram. A mobile phase comprising phosphate buffer and acetonitrile in a ratio of 58:42 with flow rate of 1.0 ml/min. A constant 30°C was maintained. The ideal wavelength for memantine and donepezil was 294 nm. Memantine's and donepezil's retention times were discovered to be 2.190 and 2.968 minutes, respectively. The percentage RSD for memantine, donepezil was determined to be 0.9 and 0.3, respectively. For memantine and donepezil, recovery was obtained at 99.94% and 99.84%, respectively. Regression models for memantine and donepezil yielded LOD and LOQ values of 0.07 ppm, 0.20 ppm and 0.01 ppm, 0.04 ppm, respectively. The regression equations for memantine (y = 9960.2x + 51.857) and donepezil (y = 9514.8x + 793.79).

Keywords: Donepezil, Memantine, Rp HPLC, Validation, Method Development.

INTRODUCTION

Memantine and donepezil are two pharmacological agents widely used in the management of Alzheimer's disease (AD), often in combination therapy. Alzheimer's disease, a progressive neurodegenerative disorder, is characterized by cognitive decline, memory loss, and behavioral disturbances, significantly impacting patients and caregivers.[1]

Memantine is an N-methyl-D-aspartate (NMDA) receptor antagonist that modulates glutamatergic neurotransmission by blocking excessive calcium influx caused by overactivation of NMDA receptors. This neuroprotective effect helps to mitigate excitotoxicity, a key pathological process in AD.[2]

Donepezil, on the other hand, is an acetylcholinesterase inhibitor that enhances cholinergic neurotransmission by preventing the breakdown of acetylcholine in the synaptic cleft. Improved cholinergic activity is associated with better cognitive and functional outcomes in AD patients.[3]

Clinical studies have demonstrated that the combination of memantine and donepezil provides synergistic benefits in moderate-to-severe Alzheimer's disease, improving cognition, behavior, and daily living activities compared to monotherapy.4 While generally well-tolerated, these medications may cause side effects such as gastrointestinal disturbances and dizziness, necessitating individualized treatment approaches.[5] Memantine is used to treat Alzheimer's disease. Initially approved by the FDA in 2013. Memantine blocks the effects of glutamate, a neurotransmitter in the brain that leads to neuronal excitability and excessive stimulation in Alzheimer's Disease. It is also chemically written as 3,5-dimethyladamantan-1-amine.**[6]** Donepezil is a medication used to treat symptoms like confusion, agitation, and memory loss associated with Alzheimer's Disease. Its is written as 2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one.[7]

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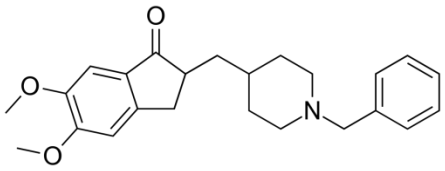
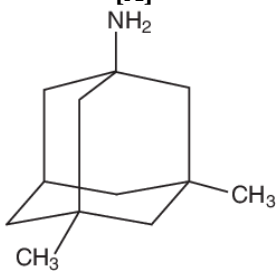


[A]



[B]

Figure 1 and 2 structure of [A] Donepezil and [B]Memantine

Numerous documented analytical processes, including the identification of more cost-effective methods, have been uncovered by extensive literature research. Therefore, a dependable and economical method for evaluating the stability of memantine, donepezil, and their pharmaceutical dosage form utilising RP-HPLC.[8-11] needs to be established and verified in accordance with ICH criteria.

Materials and Methods: Memantine and Donepezil pure pharmaceuticals (API) are available as gift samples from Spectrum Pharma Research Solution. And combination of both drug Donamem was brought from the Pharmacy. The chemicals and buffers used in this estimation were supplied by Rankem, an Indian source.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to ful fill ICH standards, we need to design and test an HPLC technique that can detect Donepezil and Memantine in pharmaceutical formulations at the same time.

Table 1: Chromatographic Conditions

Mobile phase	Disodium phosphate: Acetonitrile (42:58A)
Flow rate	1.0 ml/min
Column	Agilent C18 Column, 5 µm, 4.6 x 150 mm
Detector wave length	294 nm
Column temperature	30°C
Injection volume	10µL
Run time	10.0 min

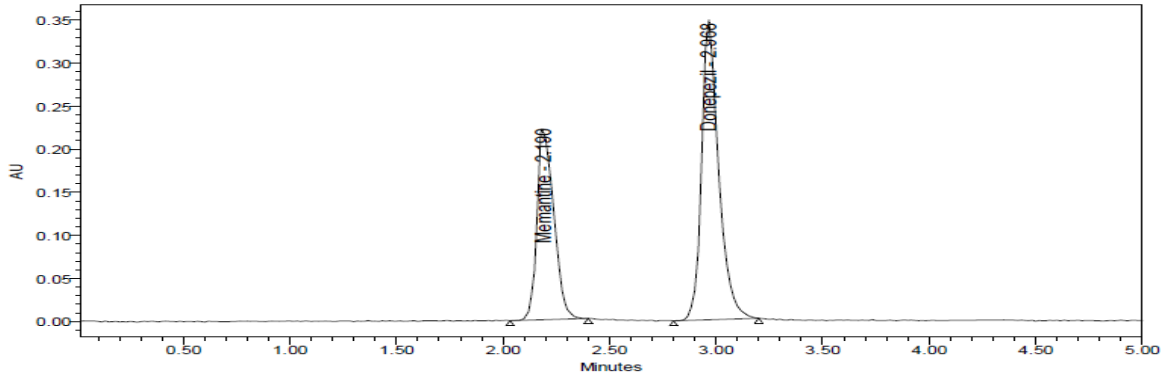


Figure 3: Optimized Chromatogram

Preparation of Standard stock solutions: 7mg Memantine and 10mg donepezil and transfer to 50ml VF. Three-fourth add of diluents sonicate of 10 min. and makeup with diluents. After that, take a 1 ml pipette out, put it in a 10 ml VF, and apply makeup using diluent.

Preparation of Sample stock solutions: 10 tablets were gauged and the normal load of every tablet was determined, then, at that point, the weight comparable to 1 tablet was moved into a 100 ml volumetric flagon, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent. Then 0.5ml sol pipette out, put it in a 10 ml VF, and apply makeup using diluent.

System suitability parameters: The system appropriateness characteristics, including peak tailing, resolution, and USP plate count, were assessed after six injections of standard solutions. An RSD of no more than 2% should be present in the region of six standard injection results.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S.no	Memantine			Donepezil			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.189	2186	1.46	2.960	6377	1.30	4.6
2	2.190	2090	1.45	2.966	6256	1.28	4.7
3	2.190	2265	1.50	2.967	6323	1.28	4.7
4	2.190	2351	1.39	2.967	6296	1.28	4.7
5	2.192	2281	1.49	2.968	6555	1.26	4.8
6	2.201	2246	1.47	2.973	6737	1.29	4.7

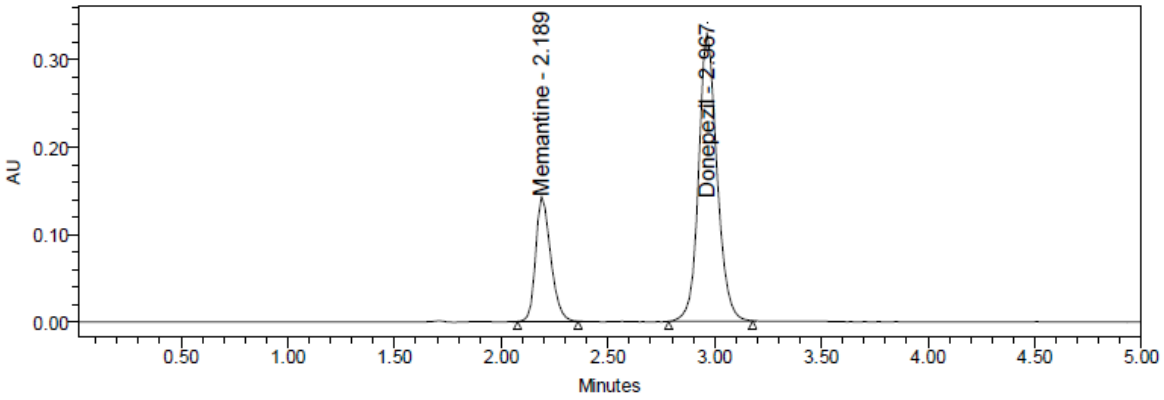


Figure 4: System suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area
Memantine	2.190	158804
Donepezil	2.968	193724

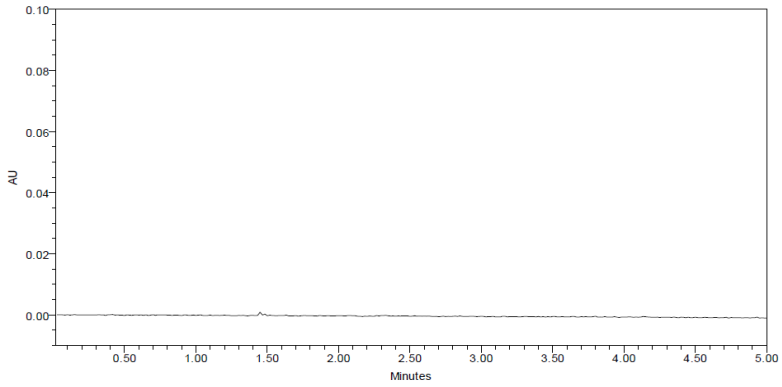


Figure.5 Blank

Force Degradation Studies: table 4 shows degradation conditions and table 5 shows the obtained degraded data and purity plot chromatogram in figure 6, 7.

Table 4: degradation conditions

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxdation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

Table 5: degradation data

Type of degradation	Memantine			Donepezil		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	1081567	93.32	6.68	392547	93.45	6.55
Base	1096857	94.26	5.74	401857	94.06	5.94
Peroxide	1103924	97.93	2.07	404988	98.62	1.38
Thermal	1142354	98.97	1.03	408541	97.60	2.40
Uv	1143854	98.97	1.03	410578	98.35	1.65
Water	1149857	94.26	5.74	411846	93.92	6.08

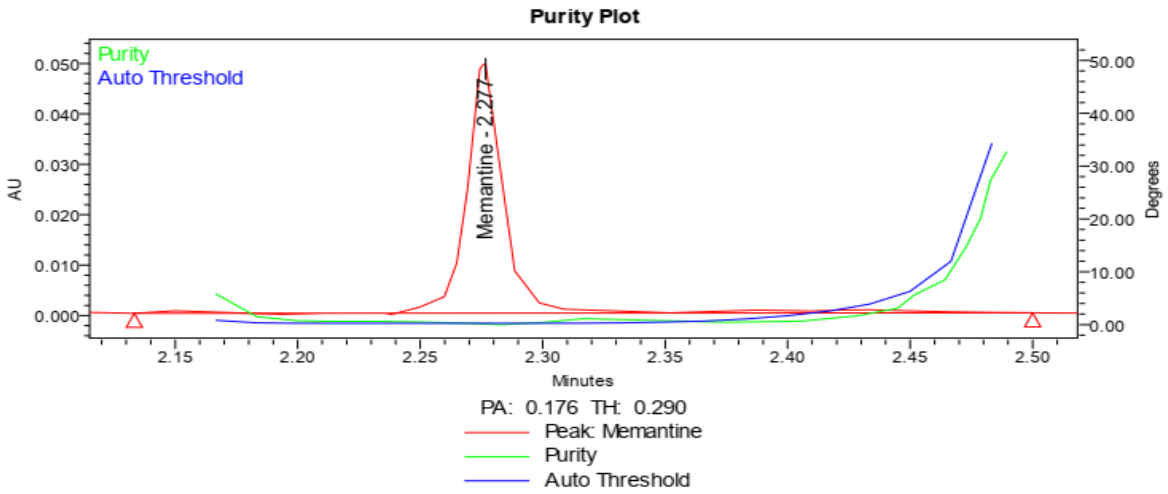


Figure 6: Purity plots for Acid Condition for Memantine

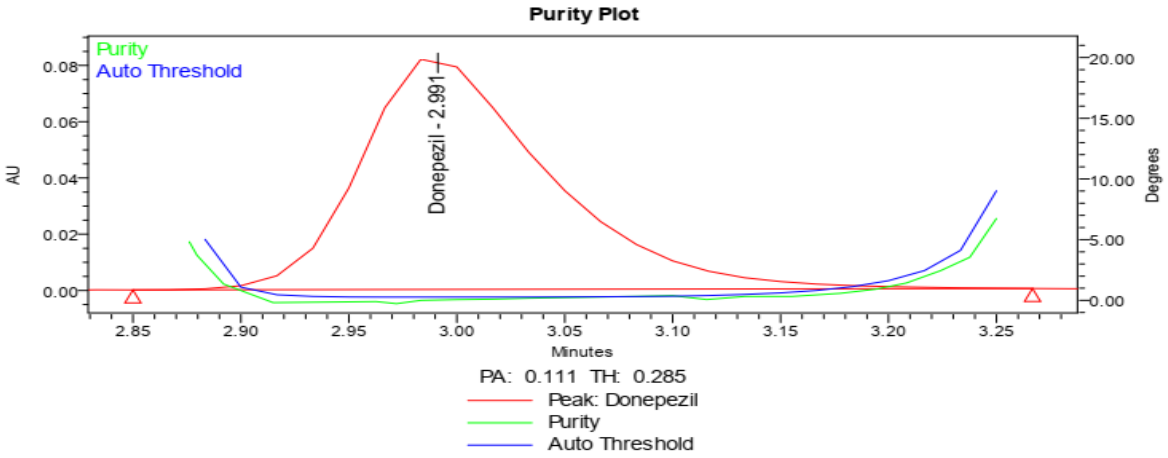


Figure 7: Purity plots for Acid Condition for Donepezil

Linearity:

Calibration data and regression data in table 6 and calibration curve in figure 8, 9

Table 6: Calibration data of Memantine and Donepezil

	Memantine		Donepezil	
S. no	**Conc (µg/mL)**	**Peak area**	**Conc(µg/mL)**	**Peak area**
1	0	0	0	0
2	3.5	34462	5	48224
3	7	68043	10	95762
4	10.5	108821	15	144253
5	14	137960	20	192808
6	17.5	174349	25	239228
7	21	208802	30	284342
Concentration range	**3.5-21**		**5-30**	
Regression Equation	**y = 9960x + 51.857**		**y = 9514.8x – 793.79**	
Co-relation	**0.9993**		**0.9999**	
LOD	**0.01**		**0.07**	
LOQ	**0.04**		**0.20**	

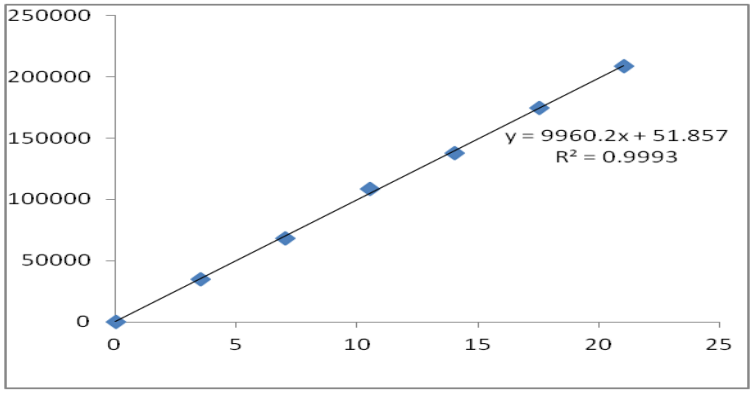


Figure 8 Calibration curve of Memantine

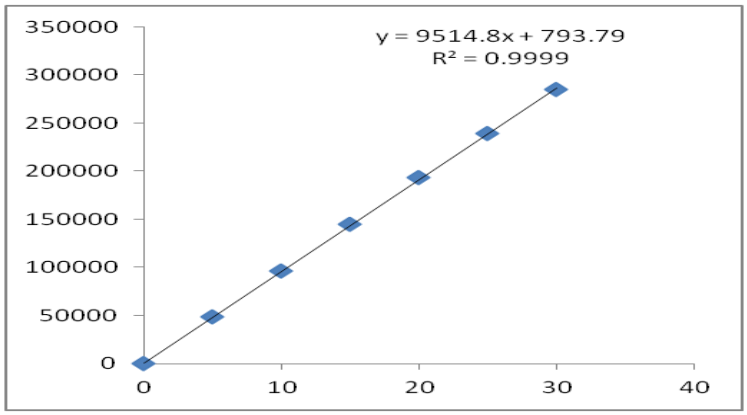


Figure 9 Calibration curve of Donepezil

Accuracy:

Recovery data shown in table 7

Table 7: recovery data of Memantine and Donepezil

	Memantine			Donepezil		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	7	7.00	100.06	10	9.91	99.12
	7	7.04	100.51	10	10.03	100.33
	7	6.98	99.66	10	10.05	100.45
100%	14	14.10	100.73	20	20.01	100.05
	14	13.92	99.41	20	20.03	100.13
	14	14.00	99.98	20	19.88	99.38
150%	21	20.83	99.19	30	29.85	99.49
	21	20.84	99.22	30	30.03	100.09
	21	21.15	100.72	30	29.86	99.53
% recovery	**99.94**			**99.84**		

System precision was performed and the data was shown in table 8

Table 8: System precision of Memantine and Donepezil

S. No	Area of Memantine	Area of Donepezil
1.	138353	193373
2.	136940	192380
3.	135636	193699
4.	138804	193737
5.	136671	193724
6.	138570	192479
Mean	137496	193232
S.D	1268.7	636.9
%RSD	0.9	0.3

The % RSD for the peak areas of Memantine and Donepezil obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Memantine and Donepezil and shown in table 9.

Table 9: method Precision

S. No	Area of Memantine	Area of Donepezil
1.	137221	193920
2.	136682	193550
3.	138346	192755
4.	137436	195000
5.	136947	192755
6.	137927	194367
Mean	**137427**	**193725**
S.D	**620.1**	**893.1**
%RSD	**0.5**	**0.5**

From the above results, the % RSD of method precision study was within the limit for Memantine and Donepezil.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (53A:47B), mobile phase plus (63A:37B), temperature minus (27°C) and temperature plus (33°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 10: Robustness data for Memantine and Donepezil.

Condition	%RSD of Memantine	%RSD of Donepezil
Flow rate (-) 0.9ml/min	1.2	0.2
Flow rate (+) 1.0ml/min	1.3	0.1
Mobile phase (-) 53A:47B	0.5	0.1
Mobile phase (+) 63A:37B	0.2	0.2
Temperature (-) 27°C	0.5	1
Temperature (+) 33°C	0.7	0.5

Assay: The API is used for standard preparations, while formulation is used for sample preparations. Six homogenous samples are injected with the sample and standards. The average percentage assay for memantine and donepezil was determined to be 99.75% and 100.05%, respectively.

Table 11: assay data

	Memantine			Donepezil		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	138353	137221	99.60	193373	193920	100.16
2	136940	136682	99.21	192380	193550	99.96
3	135636	138346	100.42	193699	192755	99.55
4	138804	137436	99.76	193737	195000	100.71
5	136671	136947	99.40	193724	192755	99.55
6	138570	137927	100.11	192479	194367	100.39
Avg	**137496**	**137427**	**99.75**	**193232**	**193725**	**100.05**
Stdev	**1268.7**	**620.1**	**0.450**	**636.9**	**893.1**	**0.46**
%RSD	**0.9**	**0.5**	**0.5**	**0.3**	**0.5**	**0.5**

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10. Formula

CONCLUSION:

The economical method developed for the simultaneous measurement of memantine and donepezil using high-performance liquid chromatography (HPLC) proved to be accurate, precise, and dependable. This method demonstrated excellent linearity, sensitivity, and repeatability, ensuring precise measurement of both drugs in pharmaceutical formulations. Because of its simpler method and reduced cost, it is ideal for routine quality control and batch release in industrial settings. In compliance with ICH guidelines, its suitability for the intended usage was confirmed.

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